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The Agricultural Chemical Society of Japan.

President : Umetarō SUZUKI.

The Council of the Agr. Chem. Soc. of Japan has decided to publish English Abstract of those papers appearing in the Journal in a separate form in order to facilitate the circulation in foreign countries.

Bulletin of the Agr. Chem. Soc. of Japan is published for this purpose from May 1926 monthly. The numbering begins with Vol. 2. No. 5. The earlier parts are represented by the English abstracts published in the Journal annexed to the Japanese texts.

The articles to be appeared in the Bulletin must be concise, supplied with experimental methods and data and understandable, without specially referring to the Japaneses texts. It ought, however, not exceed four printed pages as a rule. Any longer articles may be accepted according to the decision of the Council, with or without charge for exceeding pages.

Journal of the Agr. Chem. Soc. of Japan will be published in Japanese as formerly. Those desiring the detailed information of the articles appeared in the Bulletin may look for in the Journal of the same Number or the same Volume.

NOTE ON THE EXTRACTION OF SILK-FIBROIN BY FORMIC ACID.

By Tokuhei Kametaka.

(Received May 24 th., 1926)

I. Extraction and Purification.

Silk-fibroin is somewhat soluble in pure formic acid, but it is not so easily soluble as is given in many literatures (Baumann u. Diesser, C. 1911, I, 442 among others). This may be clearly seen from the following example of the extraction of fibroin by formic acid. 4.8g. of silk–fibroin and 50g. of pure formic acid, Kahlbaum, were heated together in a flask with reversed condenser in an oil bath of about 110° for 3 hours. Fibres gelatinized and partly dissolved. The solution was filtered from undissolved residue by water pump, and then the formic acid was distilled off almost completely under reduced pressure at about 60°. To a little brown residue absolute alcohol was added and rubbed with glass rod; white amorphous substance separated, which was filtered and dried in a vacuum desiccator. This first extract weighed 2.2g, or 44% of the original fibroin.

Insoluble residue after first extraction was similarly extracted with 25g. formic acid, and the second extract was 1.3g. or 27%. Similarly, the third extract was 0.9g. or 19%, and the residue was 0.3g. or 6 %. Thus even after three extractions still 6 % insoluble residue remains.

These extracts, when treated with hot water, separate into two parts, one soluble and the other insoluble in water. The solution was decolorized with animal charcoal, in which about half the substance is adsorped and lost, and the solution was evaporated on water bath almost to dryness and separated by adding absolute alcohol. This is the only method of purification.

II. Properties and Molecular weight of the Soluble Part.

It is white amorphous powder, and is almost all soluble in hot–water, but a small quantity of flocculent insoluble particles, into which the soluble part seems to transform on standing, always remains. The solution, when saturated with ammonium sulphate, precipitates casein–like substance.

Heated in a capillary tube, it blackens at about 200°, and decomposes at about 240°.

The substance contains very little free amino–nitrogen, 0.62% as determined by formol–titration; but after hydrosis by boiling with alkali and then neutralizing, free amino–nitrogen increased to 8 %.

Following Herzog and Kobel,[1] molecular weight determinations by freezing method, resorcin as solvent, were tried, with following results:–

(1) K. O. Herzog und M. Kobel, Ztschr. f. physiol. Chem. **134** 296—299, 1924.

Silk-fibroin 210 (Herzog and Kobel found 200),
Extract by formic acid, Insoluble Part 240,
" " " " , Soluble Part 253.

While association-products of two molecules of anhydride of dipeptide from glycine and alanine, the substance assumed by Herzog(2) as the main constituent of fibroin, is $(C_5H_8N_2O_2)_2=256$.

Owing to the lack of material, the constituent amino-acids of the soluble part could not be determined by hydrolysis. But the substance showed very faint Millon's reaction. So probably it contains no tyrosine, and may be composed only of glycine and alanine.

III. Elementary Analysis of Soluble Part.

As the substance was heated with animal charcoal, dissolving out its mineral contents, and finally precipitated by absolute alcohol, it contains more ash (3-4%) than fibroin itself, and in spite of much effort ash-free substance conld not be obtained.

Four analysis gave following average values (ash being diminished).

C 41.30, H 6.91, N 18.48

while fibroin itself gave

C 48.74, H 6.42, N 18.86

Too low value of carbon for soluble part is noticeable.

THE INVESTIGATION OF FOOD AT THE SPINNING-FACTORIES.

By Masao Shimidzu.

(From The Municipal Hygienic Labolatory of Osaka, Japan.)

(Received Feb. 10th., 1926)

Besides the theoretical studies on nutrition, it is evident that the investigation of foodstuffs actually taken by the population is also important. The author has taken up the investigation of the diet in various classes. Here will be reported the results of the author's investigations among the factory girls of three large spinning factories at Osaka.

Period of investigation.	one month each.
Average weight of body.	43.8 kg.
Average age.	18.
Total number of girls examined.	7052.

(2) Foot note of above literature.

The amount of nutrients and calories taken per day per capita is as follows:

protein(g)	fat(g)	carbohydrate(g)	Calories.
71.5	13.4	467.9	2336
13.0%	2.4%	84.6%	Total 100%

Classifying the proteins according to their respective origins, their percentages are as follows:

Total protein 100			
Vegetable origin	82. %	Animal origin	18. %
from rice	41. %	from beef & egg	2.2%
from barley	6.3%	from fish, etc.	15.8%
from beans	26.7%		
from vegetables	8.0%		

The amount of inorganic salts (especially CaO, MgO,) were calculated, from the kown data, but some of the particular food-stuffs were put into chemical analysis. It was found that each girl takes in, in average, 0.666g CaO and 1.154g MgO per day.

The author fed albino rats on the diet of the same ratio as that of the factories. The basal diet contained about ten different sorts of foodstuffs and the author added to it various nutrients, calcium, vitamins, various proteins, etc. to examine what kinds of nutrients are deficient.

According to the results of animal experiments, the diet seems to be sufficient in all respects, except in vitamin B, for the diet neither kept the body weight of the rats under examination, nor indicated any curative effect on the troubles caused by the deficiency in vitamin B.

The diet may therefore perhaps be deficient in vitamin B in the case of the factory girls for their average age.

ON THE COLLOIDAL MAGNESIUM SILICATE

By Hideo Kaneko.

(Received March 20th., 1926)

The author prepared magnesium silicate colloid by the interaction of sodium silicates (Na_2O,SiO_2, $Na_2O,2SiO_2$, $Na_2O.3SiO_2$, $Na_2O.4SiO_2$) prepared by the Asahi Glass Company and magnesium sulfate. Like plant oxidase, magnesium silicate colloid oxidises aromatic substances containing an ortho-dihydroxy grouping such as catechol, pyrogallol, and myricetin. This is expressed as a unimolecular reaction.

$$K=\frac{2.3}{\beta t}\left(\beta\log\frac{\varepsilon\infty}{\varepsilon\infty-\varepsilon}+\varepsilon\right)$$

ε : Extinction coefficient (measured by Natting's spectrophotometer)
ε_∞: Extinction coefficient at infinite time.

$\beta = -\frac{1000}{n}$, n : number of time in hour

K : reaction velocity constant.

t	A (Angle of rotation)	ε	K
0	56.5	—	—
1	59.0	0.00610	0.001452
2	61.0	0.01126	1403
3	62.7	0.01608	1355
4	64.6	2190	1430
5	66.1	2686	1437
6	67.4	3144	1414
24	76.0	7164	—
		mean	0.001430

Tannin solution turns rapidly brownish black in the presence of amino acid or its salt, when added with the magnesium silicate colloid. The hydrolysis of starch solution by acid is also stimulated by it.

The colloid which is buried in the soil shows a little stronger oxidising action than the one exposed to the sunbeam. The viscosity of the colloid is little influenced by the addition of potassium salt and the latter is also most absorbed of all alkali salts by the former.

Magnesium silicate colloid having the concentration between $\frac{1}{1000}$g. and $\frac{1}{2000}$g. protects to some degree the precipitation of calcium carbonate and silver chromate from their solutions.

ON THE PRODUCTION OF AMINES BY ASPERGILLUS ORYZAE.

By Masakazu Yamada and Shō Ishida.

(Received June 2nd., 1926)

The production of diamines, such as putrescine and cadaverine, have hitherto been observed in the bacterial decomposition of proteins (Ellinger, Z. physiol. Chem. **29**, 334, 1900), in the autolysis of yeast (Schenck; Wochsch f. Brau. **22**, 221–27, 1905; K. Kurono; Journ. Chem. Soc. of Japan **36**, 1127-52, 1917) and in the ergot by the action of higher fungus. (Rieländer; Sitzungber. Gesellsch. Naturw. Marburg. 5, Aug. No. 7, 1908).

Lately one of the authors contrived a very suitable method for the isolation of diamines with naphthol yellow and actually separated cadaverine in

every case and putrescine attaines from the Japanese brewery products saké, shōyu, miso and nattō. (M. Yamada; this Journ. Vol 2, 39–41. 1926).

It is well known that these products except the case of nattō are brewed by the cooperation of several bacteria, yeasts and moulds so that as the agent of amino-formation the action of moulds, especially of Aspergillus sp., ought not to be overlooked. The part played by the mould is the most important. It is used ordinally at the first stage of the various brewings in Japan, in the form of "koji".(1)

We used soy-bean as the source of protein and made Asp. oryzae No.54. develop on it in a wholly pure state. After about 16 days' culture, 1kg. of koji (water; 57.41%) was used for the isolation of amines by means of naphthol yellow method. By this process, putrescine and cadaverine were separated as their picrates,(2) each weighing 0.463g. and 0.483g, along with a large quantity of ammonia.

It can be concluded that same higher fungi also produce amines from proteins.

(1) Kōji is prepared from the steamed rice in case of saké, steamed mixture of wheat and soy-bean in case of shōyu, steamed soy-bean only in case of tamari-shōyu or steamed soy-bean with or without the addtion of rice or wheat, according to the kinds, in case of miso, upon which the mycelium of Aspergillus oryzae has been developped in every case.

(2) They were identified in each case from their melting point and also from the content of picric acid by means of Bussh's nitron reagent.

STUDIES ON SOIL PROTOZOA.

I. Influence of soil protozoa on nitrogen fixation of Azotobacter.

Keizô Hirai and Iwao Hino.

(Received June 5th., 1926)

The authors present in this paper their experiments carried out in order to ascertain the facts previously shown by Nasir on the influence of soil protozoa upon nitrogen fixation of Azotobacter.

The authors found that the nitrogen fixation of Azotobacter is generally stimulated and not inhibited in the presence of soil protozoa.

In the presence of soil protozoa, the highest fixation of nitrogen is recorded in this experiments to be 37.70% over the control plot in a sand culture. Out of fifteen experiments eleven showed a decided gain in nitrogen fiixation over the control and two showed no gain, while two gave negative results

which are only the cases when the saturated sandy soil is used.

pH value of the nutrient media always changes in the course of experiments: when azotobacter alone exist the medium considerably acidifies, and when soil protozoa coexist it slightly acidifies, while in the single existence of soil protozoa it alkalifies in all cases.

It seems to the authors that the soil protozoa and azotobacter live in the state of disjunctive symbiosis or of metabiosis in the strict sense : in other words, the presence of soil protozoa decreases the acidity of the nutrient media, resulting the vigor of growth and increased fixation of azotobacter, consequently giving favorable effect on the protozoa themselves to increase the vigorosity and multiplication, thereby keeping the active state of protozoa for a longer period.

ON THE SOME NITROGENOUS CONSTITUENTS OF THE LEAVES OF KUZU (Preliminary report) (The Japanese arrow-root plant, Pueraria hirsuta, Matsum.)

by Rinjiro Sasaki

(Received June 17th., 1926)

Many vegetables contain less nitrogen than grains and even if, sometimes, comparatively much nitrogen is contained in a vegetable, the great part of it is of non-protein nature. But there are some wild vegetables that contain comparatively much protein nitrogen. At present, the nutritive value of vegetables is thought to be depend on their mineral matters (ash) and various kinds of vitamins, and the value of the nitrogenous material is almost neglected.

Various green forage plants contribute appreciable amounts of protein to the ration of farm animals, but practically nothing is known of the chemistry of these, and even the proportion of protein in these plants is not yet established.

In our laboratory the white rats were fed on polished white rice or barley, each added only fresh vegetables, without other supplements. These rats maintained normal growth and nutrition and in some of them two generations succeeded. It is the known fact that the white rat fed exclusively on polished rice can not grow normal for the deficiency of ash and vitamins and also probably of protein. The author thinks, the above mentioned normal growth is resulted from the supply of good nitrogenous materials, as well as of the mineral matters and vitamins from vegetables.

A serious gap exist in our current knowledge of the chemistry of nutrition which makes it impossible to apply to the practical problems of feeding on the farm what has been learned of the nutritive value of the proteins of the cereals as well as of the protein concentrated.

There are not so many investigations referring to the nitrogenous constituents of green forage.

The Japanese arrow-root plant *KUZU* (Pueraria hirsuta, Matsum.) are a leguminous plant and grow wild everywhere in Japan. The leaves contain fairly much nitrogen and are used as fodder. In preparing the hay, the leaves are easy of drying in the air and difficult of dropping from the stem. When it is mixed to the other fodders, it stimulates appetite, so it is usually thought as having special action for maintaining of health of farm animals.

The investigation reported preliminary in this paper embodies attempts to examine the nutritive value of vegetables and also the chemical properties of the protein of the arrow-root leaves.

The wild green arrow-root plant was harvested at the time of full bloom and dried up in the direct sunlight and preserved.

(I) General composition

The general composition was examined by the usual method.

Table I

General composition of arrow-root leaves

Water (%)	Dry matter (%)	In dry matter (%) Crude Protein	True Protein	Ether Extract	Crude Fiber	Crude Ash	Nitrogen-free Extract
10.34	89.658	18.148	17.262	4.799	24.915	8.278	43.860

In 100 Parts of Crude Ash (%)

SiO_2	SO_3	P_2O_5	Mn_3O_4	CaO	MgO	Fe_2O_3	Al_2O_3	Na_2O	K_2O	Undetermined	Cl
11.080	1.935	5.086	0.583	39.071	6.278	0.790	1.340	11.002	20.954	0.873	1.302

(II) Solvents for extraction of nitrogenous constituents.

As it will beseen from Table I, the leaves of arrow-root plant contain much protein. Naturally we must employ the best solvent or apply the best treatment for the isolation of the protein. The author examined various solvents to select the most suitable one for the extraction of nitrogenous matter. 250 ccm. of each of these solvents were applied to the different lot of 5 grm. of the same sample and the extraction durated for 24 hours. In the case of the alkaline alcohol solvent, it was boiled for five minutes with shaking from time to time, and the total nitrogen was estimated in 100 ccm. of liquid. The results are shown in Table II.

Table II

The amounts of nitrogen extracted by various solvents.

	In dry matter (%)	In total N (%)
Total N	2,904	——

Protein N	2.762	95.117
Non-protein N	0.142	4.883
Water soluble N	0.375	12.915
10% NaCl 〃 N	0.368	12.684
5 % CH_3COONa 〃 N	0.454	15.646
0.2 % NaOH 〃 N	0.689	23.742
75 % alcohol 〃 N	0.134	4.608
60 % alcohol containing 0.3 % NaOH soluble N	1.732	59.689
Ether soluble N	0.015	0.526
Water soluble N in the residue of ether extraction	0.343	11.826
60 % alcohol containing NaOH soluble N in the residue of ether and water extraction	0.305	10.515

(**III**) Extraction and preparation of nitrogenous constituents from the leaves.

The powdered leaves of the arrow-root plant (500 grms.) were extracted by boiling with 60 per cent alcohol containing 0.3 per cent NaOH. The liquid was separated by the filter cloth. The resulting extract was filtered through paper pulp, which removed a small amount of soil material and the green colloidal substance. The filtrate was neutralized by the addition of dilute HCl, but there was no appreciable precipitate. Then this solution was concentrated to about 2000 ccm. in vacuo. To this concentrated solution which contained a small amount of precipitate was added the requisite amount of HCl to effect a complete precipitation. The precipitate was amorphous and was insoluble in excess of HCl. This was separated by centrifuging. It was redissolved by adding dilute NaOH and filtered through paper pulp, and the clear but brown colored solution was obtained. The addition of the requisite amount of HCl to the filtrate caused the precipitate to separate. Thus purified, the precipitate was centrifuged off, washed with distilled water acidified with small amount of HCl, and dried in the air. The weight, moisture-free, was 30 grms. and the yield of nitrogenous matter was 34 per cent of the total protein nitrogen.

(**IV**) Chemical properties.

This is insoluble in either dilute or strong HCl, but soluble in small excess of NaOH to give a clear yellowish brown solution. This is insoluble in ether and alcohol. The precipitate is produced on adding dilute acetic acid to the alkaline solution and it dissolves to an opalescent solution in excess of acetic acid, and the precipitate is not produced on dilution. When calcium chloride solution is added to an alkaline solution, a brown precipitate is formed. The mixture of glacial acetic and conc. HCl dissolves completely the precipitate, but on dilution with water the precipitate is again produced.

Millon's, Adamkiewicz's, Molish's reaction and Xanthoproteic reaction

did not distinctly appeared by the abstraction of pigment. The Ninhydrin reaction appeared clearly in the acid-hydrolyzed solution.

(**V**) Analysis of the precipitate.

The moisture-free precipitate contains 14.153 per cent of nitrogen, 0.58 per cent of ash and some of phosphorus. The nitrogen content, ash-free, is 14.236 per cent. Molish's and furfurol reaction were positive, showing the presence of carbohydrate in the preparation, but the author have not, as yet, had an opportunity to determine whether it is conjugated or impurity. Table III gives the distribution of nitrogen by van Slyke's method after hydrolysis for 48 hours with 20 per cent HCl.

Table III

The distribution of nitrogen

Total nitrogen used for analysis was 0.3500 grm.

	Nitrogen (grm.)	In total N (%)
Amide N	0.0144	4.11
Humine N	0.0969	27.69
Total N in Phosphotungstate Precipitate	0.0545	15.57
Amino N 〃 〃 〃	0.0206	5.89
Arginine N	0.0308	8.80
Cystine N	0.0005	0.14
Histidine N*	0.0162	4.63
Lysine N	0.0070	2.00
Total N in Filtrate (Mono-amino acid)	0.1685	48.14
Amino N in Filtrate	0.1219	34.83
Total N recovered×	0.3343	95.51
Total N of Monoamino-dicarboxylic acid (Total N used for analysis was 0.3212 grms.)	0.0866	2696

*The formula is: Histidine N = 1.5 Total non-amino N − 1.125 Arginine N.

× Andersen-Müller's procedure was adopted.

(**VI**) An unknown crystalline nitrogenous substance.

The filtrate obtained from the precipitate produced by adding dilute HCl to the concentrated extract of the sample (500 grms. of leaves), was concentrated to about 2000 cc. in vacuo and was filtered again through a paper pulp.

The transparent dark brown filtrate thus obtained was allowed to settle in the cold. The crystalline precipitate was produced. This was separated by centrifuging from mother liquid, washed with the mixture of absolute alcohol and ether, and dried. The precipitate purified in this way represents light greenish tetragonal prisms, melting at 185—187°C, and was completely soluble in NaOH, giving a deep yellow brown solution. The crystal is soluble in acetone and dilute alcohol but not in ether and absolute alcohol. In an acetone solution it is precipitated by the addition of NaOH. In an alkaline solution it is precipitated by the addition of $CuSO_4$. Millon's and Xanthoproteic reaction are positive.

ON THE COMBUSTION TEMPERATURE OF CIGARS AND CIGARETTES.

By Masuzo Shikata.

(Received Apr. 11th., 1926)

It is rather noticeable, that, although so many analysis have been carried out as to the chemical composition of smoke of cigar, no attempt seems to have been undertaken for a measurement of actual combustion temperature of cigars and cigarettes.

The present author has measured the temperature of combustion with a small enamelled iron-constantan thermocouple with its diameter of 0.14 mm.

This thermocouple, when inserted directly in the axial center of cigars and cigarettes can give actual temperature of such small body as cigarette, owing to its very small heat capacity. The calibration of this thermocouple has been done with melting points of metallic tin, zinc and antimony.

The supply of air, of course, is one of the most important factors of combustion, that is to say, smoking, or better to say, sucking of cigarettes is one of the decisive factors for the combustion temperature.

Special cares having been payed in this respect, the measurements have been carried out.

The following table shows the stable maximum temperatures, i. e. maxima of stable temperature without sucking or with very slow sucking.

Table .

Numbers of experiments	Asahi	Shikishima	Golden Bat	Star	Westminster	Cigar
1	515	733	649	587	649	597
2	473	671	583	420	753	469
3	552	609	557	649	743	671
4	463	597	597	701	738	629
5	582	582	672	567	701	687
6	530	577	493	592	733	
7	619	520	649	671	749	
8	597	567	602	619	748	
9	711	557	530	723	671	
10	614	676	733	691	795	
Mean	565.8	608.9	606.4	622	726.5	646.6

In the table, the first line is the names of cigars and cigarettes.

Asahi, Shikishima, Golden Bat and Star are Japanese cigarettes prepared by the Japanese Tobacco Monopoly Bureau. The cigar is "Orientales", also from the Monopoly Bureau. Westminster is Turkish Blend A. A. Grade,

prepared by Westminster Tobacco Co. Ltd. London.

In Fig. 1. (in the original paper) the method of measurement has been graphically shown. In Fig. 2. the temperature change with time is manifested. Temperature is given by ordinate, time by abscissa in minutes. In this diagram process of combustion are clearly seen.

In case of the cigar the temperature increases gradually.

Such condition must be favourable for the distillation of nicotin, nicotein and other essential components.

In case of cigarettes the maximum temperature is attained suddenly, so that heat decomposition must set in, before most parts of essential components can be distilled or sublimated. It can be noticed that cigarettes of Westminster Tobacco Co. shows much higher combustion temperature than ordinary cigar, and latter has again higher combustion temperature than the Japanese cigarettes.

ON THE PRESENCE OF CYSTEINE GROUP IN PROTEIN MOLECULES.

By Yuzuru Okuda.

(Received May 29th., 1926)

The purpose of this investigation was to make some quantitative studies concerning the presence of cysteine group in the protein molecule, a subject on which, as far as we know, no work has hitherto been done.

I. Is Cysteine Produced Secondarily from Cystine during Prolonged Hydrolysis of Proteins?

Mörner[(3)] has hydrolyzed some horn substance with hydrochloric acid for a week, and after removing the most part of the acid by evaporation, neutralized the hydrolysate with lead oxide, treated with hydrogen sulphide, and then filtered the precipitate, and after perfectly removing the excess of hydrogen sulphide tested cysteine in the filtrate by means of the nitroprusside reaction. And he has stated that cysteine is produced secondarily from cystine during prolonged hydrolysis of the protein. But we have failed to confirm his conclusion. We have repeated his procedure with pure cystine and with some keratine–cleavage–products free from cysteine, and have verified the facts that by Mörner's process cysteine should be produced from cystine by the reducing

action of hydrogen sulphide, but that cysteine is not produced from cystine during prolonged hydrolysis and on the contrary cysteine, in the course of hydrolysis, gives rise to cystine, and that both cysteine and cystine are the primary products of the complete hydrolysis.

II. The Qualitative Test of Cysteine in Proteins.

Heffter[2] and Arnold[1] have demonstrated that some proteins give the nitroprusside reaction while others do not. We repeated their experiments with various proteins, directly or after the treatment with enzymes and oxidizing agents, and have confirmed their findings, and came to the conclusion that there are sulphur linkages, such as R–SH, R–S–R and R–S–S–R, in protein molecules. The protein containing R–SH group gives the reaction directly, the one containing R–S–R group indirectly or after a certain degree of hydrolysis, and the other containing R–S–S–R alone does not.

III. Presence of Cysteine in the Protein–hydrolysates.

For the purpose to show the presence of cysteine in the hydrolysate of proteins, cysteine and cystine were determined, by means of the iodine–method[4], in the several stages of hydrolysis of proteins. Egg albumine and wool gave only a minute quantity of cysteine in comparison with that of cystine but muscle–proteins freshly prepared gave much cysteine, for instance, the muscle–protein of pagrus major, when hydrolyzed with hydrochloric acid in the current of carbon dioxide gas, gave nearly an equal amount of cysteine and cystine as shown in the following table.

Hour of hydrolysis	Cysteine	Cystine	Ratio		Cysteine-reaction	Remarks
			Cysteine	Cystine		
3	33.8	36.1	94	100	+	Hydrolyzed and treated in CO_2 gas.
5	34.1	36.4	94	100	+	
7	34.8	37.4	93	100	+	
20	0.0	98.4	0	100	–	In the air.

When it is borne in mind that cysteine is easily oxidizable to cystine, during the preparation of proteins, and especially in the instant of the cleavage of protein molecules, the cysteine content of living muscle–proteins should be predominate to their cystine content.

IV. The Effect of Acid Hydrolysis upon Cysteine.

For the purpose to know the rate of oxidation of cysteine to cystine, the effect of acid hydrolysis upon cysteine was studied with pure cysteine and with a mixture of cysteine and gelatine which contained no cysteine. The hydrolysis was performed in the usual way or in the air. The quantity of performed cysteine and cystine produced were determined quantitatively. The result of an experiment performed with a mixture of cysteine and gelatine was as follows :—

Hours ef hydrolysis	Cysteine found	Cystine found	Rate of oxidation (%)
2	0.90	0.21	18.6
4	0.86	0.24	22.1
6	0.78	0.33	30.0
8	0.73	0.38	33.8
10	0.67	0.44	39.4
15	0.65	0.47	41.5
25	0.60	0.51	45.5
30	0.56	0.55	49.3
54	0.36	0.75	67.1
82	0.14	0.95	87.8
100	0.00	1.01	100.0

In this case, the oxidation was slow and the cysteine reaction disappeared after about 100 hours of hydrolysis, but when muscle-proteins containing the cysteine group was treated in the same way the oxidation was more rapid, and after 20 hours the cysteine reaction was negative. From these experimental results we see that isolated cysteine is more stable than cysteine in the nascent state which is reactive and is easily oxidizable to cystine in the instant of the cleavage and during the hydrolysis, and that when cysteine was boiled with hydrochloric acid as mentioned above it became cystine quantitatively.

Conclusion.

So far as these experimental results are concerned, we came to the following conclusions :— During prolonged hydrolysis of proteins, cysteine is not produced from cystine but on the contrary cysteine gives rise to cystine. A cysteine group is present in some protein molecules and cysteine found in the hydrolysates. Both cysteine and cystine are the primary products of the complete hydrolysis.

My thanks are due to Mr. Y. Nishijima, my assistant, for his analytical work.

Reference.

(1) Arnold: Zeitschr. f. physiol. Chem., **70**, 300, 1910—11.
(2) Heffter: Jahrber. ü. d. F. d. Tierchem. **37**, 565.
(3) Mörner: Zeitschr. f. physiol. Chem., **28**, 595, 1899.
(4) Okuda: J. Departm. of. Agr. Kyushu Imp. Univ., **1**, 163, 1925.

SUR LES PRODUITS DE LA FERMENTATION DU MONASCUS PURPUREUS (CHAMPIGNON DE L'ANG-QUAC).

Par Yusuke Sumiki.
(Collège de chimie agricole de l'Université de Tokyo).

(Received June 5th , 1926)

Le genre Monascus a été classifié en deux espèces, cést-à-dire Mon. ruber et Mon. mucoroides par Van Tieghem. (Bull. de Soc. Bot. de France,

31,1884.)

F. A. Went a isolé une nouvelle espèce du champignon de l'ang-quac et a donné à cause de sa couleur pourpre le nom Mon. purpureus. Il a étudié sa morphorogie, son développement et sa nourriture. (Ann. Soc. Nat. Bot., 8, 1, 1895.)

Prinsen Geerlig a fait une étude des propriétés chimiques de matière colorante de l'ang-quac. (Chem. Ztg., 19,1311, 1895.)

Ueda a prouvé l'existence du Mon. purpureus dans "Beni-koji" de l'Anchû. (Tokyo Bot. Mag., 1902,)

S. Ikeno (Ber. Deut. Bot. Ges., 11,259,1903.) et H. Kuyper (Ann. Myco., 3,32,1905.) ont étudié sur les divers points le développement de périthèces et la formation de spores.

Récemment S. Hagiwara a étudié les enzymes et la matière colorante de ce champignon. (Taiwan Kôgyôbu Hôkokusho, 5,1924.)

EXPERIENCE. I. Les produits de la fermentation.

J'ai cultivé le Mon. purpureus à la température de 26—30° pendant 14—50 jours dans l'extrait kojique ou dans une solution nutritive suivante:

Glucose ou sucre de canne	10g.	KH_2PO_4	0.015g.
Peptone ou KNO_5	0.1—1g.	K_2HPO_4	0.015g.
$MgSO_4$	0.01g.	$CaCl_2$	0.010g.
$FeCl_3$ et NaCl	trace.	Eau distillée	100g.

On distille la solution cultivante de ce champignon à la vapeur d'eau. Après l'évaporation du résidu, on l'acidifie et l'extrait avec l'éther. On obtient l'acide succinique et l'acide lactique de cette solution éthérée (A).

On ajoute l'excès du carbonate de barium dans ce distillatum à la vapeur d'eau et le chauffe dans le ballon fourni d'un réfrigérant descendant, on filtre le contenu du ballon et distillele filtratum à la vapeur d'eau encore une fois. On obtient un distillatum(B) contenant de l'alcool éthylique, de l'aldéhyde acétique et de l'huile de fusel.

Après l'évaporation du résidu de la distillation à la vapeur d'eau, on obtient des sels de barium des acides volatils (C); acides formique et acétique.

Présence de l'alcool éthylique.

Par la répétition des distillations fractionnées du distillatum (B), on obtient une fraction 78°, l'on ajoute l'oxyde de calcium pour échapper de l'eau. On chauffe avec la quantité théorique du isocynate de phenyl au bain-marie dans le tube fermé. On obtient des crystaux longues aiguilles de phenyl uréthane fondant à 50—52°.

0.0924g. Subst. ont donné 7.1cm³	N_2 (24°, 758m.m.).
Calculé pour $C_9H_{11}O_2N$	N 8.49%.
Trouvé	N 8.55%.

Présence de l'huile de fusel.

On trouve la présence de l'huile de fusel dans le distillatum (B) par les réactions suivantes:

Réaction de T. Takahashi (Ztschr. f. N. u. G., 27,820,1914.).

Réaction de Komoroski (Chem. Ztg., 27,808,1903.).

Réaction de Udrannszky (Ztschr. f. Phy. Chem., 13,261,1888.).

Présence de l'aldéhyde acétique.

Par la distillation fraetionnée du distillatum(B), on obtient une fraction 50—78° qui donne une couleur rouge avec le réactif de Schiff.

Réactions:

couleur rouge avec le réactif de Schiff.

précipité jaune avec le réactif de Nessler (E. Pittarelli, Chem. Zentbl., 4,616, 1920) (jaune par l'aldéhyde; blanche par l'acéton).

réduction de la solution de Fehling.

réduction de nitrate d'argent ammonical.

réaction de Jeau.

réaction de Auld et Hantzsch(Ber. Deut. Chem. Ges., 27,514,1888).

réaction de E. Pittarelli(Jour. Pharm. Chem., 23,21,1921).

On ajoute du dimedon dans le distillatum obtenu et obtient des crystaux blancs de l'acétaldomedon fondant à 137—9°. Mélangé avec l'acétaldomedon pur, il fond à la même température.

Présence de l'acide succinique.

Aprés distillation de l'éther de la solution éthére(A), on obtient des crystaux prismatiques fondant à 183° par récristallisation dans l'eau bouillante.

0.1482g. Subst. ont donné 0.2202g. CO_2 et 0.0705g. H_2O.

Calculé pour $C_4H_6O_4$ C 40.67% H 5.08%.

Trouvé C 40.52% H 5.28%.

Présence de l'acide lactique(racémique).

Par le traitement avec le carbonate de calcium, on obtient des crystaux longues aiguilles de lactate de calcium. Pour analyser il a été desséché à 110°.

0.4193g. Subst. ont donné 0.2675g. $CaSO_4$.

Calculé pour $C_6H_{10}O_6Ca$ Ca 18.34%

Trouvé Ca 18.62%

Par le traitement avec le carbonate de zinc, on obtient le lactate de zinc cristallisé en rhombique et on le sèche à 110° pour l'analyse.

0.2552g. Subst. ont donné 0.0845g. ZnO.

Calculé pour $C_6H_{10}O_6Zn$ Zn 26.75%.

Trouvé Zn 26.60%.

Rotation optique en solution aqueuse et eau de cristallisation du lactate de zinc:

dissout 0.4823g. Subst. dans 30g. d'eau. L=Idm. (α) = 0.

0.2296g. Subst. ont perdu à 110° 0.0412g. H_2O.

Calculé pour	$(C_3H_5O_3)_2Zn\pm3H_2O$	H_2O 18.18%	······(α)D= 0.
〃 〃 〃	$\pm2H_2O$	H_2O 12.89%	······(α)D= ±9°.
Trouvé		H_2O 17.90%.	

Présence de l'acide formique et de l'acide acétique.

On récristallise les sels de barium des acides volatils(C) dans l'eau et

sèche à 110°.

0.3765g. Subst.	ont donné 0.3412g.	$BaSO_4$.	
Calculé pour	$(HCOO)_2Ba$	Ba 60.42%.	
〃 〃	$(CH_3COO)_2Ba$	Ba 53.97%.	
〃 〃	$(C_2H_5COO)_2Ba$	Ba 48.55%.	
Trouvé		Ba 53.42%.	

On acidifie les sels de barium et distille. On trouve la présence de l'acide formique dans ce distillatum avec les réactions suivantes:

réduction de la solution du $HgCl_2$(précipité blanche du $HgCl$).

réduction de nitrate d'argent ammonical(miror d'argent).

On ajoute l'oxyde mercurique jaune dans ce distillatum et chauffe pour décomposer de l'acide formique, on filtre, on évapore doucement.

On récristallise dans l'eau et sèche dans vide sur de l'acide sulfurique jusju'à poids constant.

0.6226g. Subst.	ont donné 0.4500g.	HgS.	
Calculé pour	$(CH_3COO)_2Hg$	Hg 62.96%.	
〃 〃	$(C_2H_5COO)_2Hg$	Hg 52.48%.	
Trouvé		Hg 62.43%.	

EXPERIENCE. II. Relation entre la nourriture azotée et les produits de la fermentation.

Nourriture azotée·········Les quantités differentes des $(NH_4)_2SO_4$, KNO_3, peptone et asparagine, chacun en comprenant 0.1, 0.3, 0.5 et 1%, sont dissoutes dans la solution nutritive.

J'ai déterminé de la solution cultivante fermentée:

1° L'alcool éthylique(fig. 3), par la méthode du distillation.

2° L'acide volatil (fig.5). On distille 50c.c. de la solution cultivante à la vapeur d'eau jusqu'à 200c.c. du distillatum et titre avec l'alcali titré.

3° L'acide non volatil(fig.4). On titre la solution cultivante fermentée avec l'alcali titré et diminue de cette acidité déterminée l'aciditéde l'acide volatil précédent et l'acidité primitive de la solution nutritive in-fermentée.

4° La glucose(fig.1), par la méthode de Bertrand.

5° Poids du champignon(fig.2).

EXPERIENCE. 111. Sur la zymase.

Par la méthode de macération de A. Lebedeff(Ann. de Inst. Past., 26,7,1912) J'ai obtenu le résultat positif, mais Je mettrai cette publication à l'autre jour.

CONCLUSION.

1° Comme produits de la fermentation avec ce champignon, se produi sent en grand partie l'alcool éthylique, puis l'acide succinique, ensuite l'acide lactique et l'acide acétique; en petite quantité l'aldéhyde acétique et l'acide formique.

2° Entre les quantités differentes······0.1—1%······des matière azotée la quantité la plus favorable pour produir l'alcool éthylique est de 1% de la solution nutritive. Et pour produire les acides, J'ai trouvé que 0.1% de matière azotée est la plus favorable.

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